CLAIMS

We claim:

1. A method for producing a biological substance comprising:

(a) culturing a cell culture comprising a cell culture medium and cells,

(b) perfusing the cell culture in a bioreactor with a basal medium and a feed medium, and

(c) harvesting the biological substance,

wherein the basal medium and the feed medium are fed to the cell culture at different rates, the cell culture is continuously passed through a separation system, and the cells are retained in the bioreactor without bleeding.

2. The method of claim 1, wherein the separation system is an alternating tangential flow (ATF) device or tangential flow filtration (TFF) device.

3. The method of claim 1, wherein the separation system comprises a hollow fiber filter, wherein the pore size of the hollow fiber filter is about 0.08 μm to about 0.5 μm.

4. The method of claim 3, wherein the pore size of the hollow fiber filter is about 0.1 μm to about 0.5 μm.

5. The method of claim 3, wherein the pore size is about 0.2 μm or about 0.45 μm.

6. The method of any one of claims 1-5, wherein the basal medium is fed at a perfusion rate of about 0.1 to not higher than about 2.0 working volumes per day (VVD).

7. The method of any one of claims 1-5, wherein the basal medium is fed at a perfusion rate of about 0.1 to about 1.5 (VVD).

8. The method of any one of claims 1-5, wherein the basal medium is fed at a perfusion rate of about 0.5 to about 1.0 (VVD).

9. The method of any one of claims 1-5, wherein the perfusion of the feed medium is at a rate ranging from about 0.1 to about 20% of the perfusion rate of the basal medium.

10. The method of any one of claims 1-5, wherein the perfusion of the feed medium is at a rate ranging from about 1 to about 15% of the perfusion rate of the basal medium.

11. The method of any one of claims 1-5, wherein the perfusion of the feed medium is at a rate ranging from about 1 to about 9% of the perfusion rate of the basal medium.

12. The method of any one of claims 1-11, wherein the cells are cultured at a range of about 35°C to about 37°C.

13. The method of any one of claims 1-12, further comprising subjecting the cell culture to a temperature shift to a temperature in the range of about 28°C to about 33°C.

14. The method of any one of claims 1-13, wherein the temperature is lowered before peak viable cell density (VCD) is achieved.

15. The method of any one of claims 1-14, wherein an antifoam is added to the bioreactor.

16. The method of any one of claims 1-15, wherein a microsparger is used.

17. The method of claim 16, wherein the microsparger delivers oxygen at a flow rate in a range of about 0.2 to about 0.5 VVM.

18. The method of any one of claims 1-17, wherein the cells comprise mammalian cells.

19. The method of any one of claims 1-18, wherein the biological substance is chosen from receptors, enzymes, fusion proteins, blood proteins, multifunctional proteins, viral or bacterial proteins, and immunoglobulins.

20. The method of any one of claims 1-19, wherein said method achieves an accumulative volumetric productivity (Pv) of about 10 g/L or more.

21. The method of any one of claims 1-20, wherein said method achieves an accumulative volumetric productivity (Pv) of about 15 g/L or more.

22. The method of any one of claims 1-21, wherein said method achieves an accumulative volumetric productivity (Pv) of about 20 g/L or more.

23. The method of any one of claims 1-22, further comprising subjecting the harvested biological substance to a continuous product capture process by at least one chromatography step.

24. A biological substance produced by the method of any one of claims 1-23.

25. A system for producing a biological substance comprising:

(a) a module for perfusing a cell culture in a bioreactor with a basal medium and a feed medium; and

(b) a module for continuously harvesting the biological substance, comprising a hollow fiber filter having a pore size or a molecular weight cut-off (MWCO) larger than the molecular weight of the biological substance.

26. The system of claim 25, further comprising a module for continuous capture of the biological substance from the harvested materials.

27. The system of claim 25, wherein the module for continuously harvesting the biological substance is an alternating tangential flow (ATF) device or tangential flow filtration (TFF) device.

28. The system of claim 25, wherein the basal medium and the feed medium are fed at different rates.

29. The system of claim 25, wherein the pore size of the hollow fiber filter is about 0.08 μm to 0.5 μm.

30. The system of claim 25, further comprising a bioreactor for cell culture and/or a microsparger.